THE EXPERIMENTAL PRODUCTION OF HYPOTYPICAL OVARIES THROUGH UNDERFEEDING. A CONTRIBUTION TO THE ANALYSIS OF STERILITY.[1]

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In former communications we described a condition of the ovaries in which the follicles become atretic, usually before they have reached medium size. Mature follicles are not produced under these conditions and sterility ensues which persists as long as this condition lasts.[1] Such ovaries we designated as hypotypical. We observed those changes especially in guinea pigs in which the corpora lutea had been burnt at a certain period after ovulation. We must assume that the burning of a part of the ovary caused a "tissue shock" in the remaining part of the ovary which, without killing the follicles, weakened the granulosa cells and thus prevented their further development and caused their early disintegration. In addition we found this change in a number of other animals, some of which had not yet been in heat, and had consistently refused copulation, although their age was such that we might have expected to find them sexually active. In some other cases in which as the microscopic examination showed, ovulation had failed to take place after the conclusion of the last sexual period notwithstanding the degeneration of the corpora lutea, hypotypical ovaries were likewise found. This suggests that this condition may be the cause of long-lasting or perhaps perpetual sterility. This hypotypical condition of the ovaries is of theoretical interest, inasmuch as it represents a state of low developmental energy of an organ that is normally in a condition of constant change. From a practical point of view it is of importance because it can lead to sterility of the gonads. It seemed to us therefore of interest

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[2] Leo Loeb, *Zentralblatt f. Physiologie*, 1911, XXV., No. 9; *Virchow's Archiv*, 1911, CCVI., 278.

to carry out further experiments in order to throw additional light on the causes of the hypotypical condition of the ovaries. It occurred to us that a common cause might underlie all those cases in which a local injury of the ovaries was not present, and that lack of proper nourishment might be an important factor in this form of temporary sterility. We therefore carried out four series of experiments in which female guinea pigs received the same kind of food as usual, viz., oats and a little grass or green vegetables, but both in much reduced quantity. We shall discuss our experiments in the inverse order in which they were done. In all cases but one both ovaries were cut in serial sections after the completion of the experiment. In one animal only one ovary was cut serially.

IV. Series.

Five guinea-pigs were used in this experiment. (See Table I.)

Table I.

IV. Series.

Guinea Pig.	Original Weight.	Loss of Weight in Per Cent. of Original Weight.	Period During Which Loss of Weight Occurred.	Result.
A....	294 grams	28%	12 days	Animal weak at end of experiment; about 10–13 days previously ovulation. Hypotypical ovaries of first order.
B.....	309 grams	21% (in 5 days)	6 days	Found dead. Good-sized follicles in granulosa-Degeneration, preparatory to the development of hypotypical ovaries. No ovulation.
B_1....	288 grams	27%	10 days	Weak, killed. Hypotypical ovaries of second order. Delayed ovulation.
C.....	331 grams	36%	14 days	Weak, killed. Hypotypical ovaries of second order. Delayed ovulation.
D.....	342 grams	34%	14 days	Weak, killed. Necroses in liver. Hypotypical ovaries of first order, delayed ovulation.

Microscopic Examination.

Guinea Pig A.—Had lost 28 per cent. of its weight twelve days after beginning of feeding experiment.

In the ovaries: small and small to medium follicles. In the

latter slow disintegration of granulosa cells takes place and connective tissue grows in. Small follicles in various stages of connective tissue atresia; many follicles in the last stage of atresia, with relative prominence of the theca interna. Remnants of old retrogressing corpora lutea: yellowish stained vacuolar cells surrounded by dense hyaline fibrous tissue, traversed by thick-walled blood vessels. In addition we find well-preserved small corpora lutea with capillaries and myxoid connective tissue in center. Mitoses are not present in the corpora lutea. The *uterus* thin, with low cylindrical surface epithelium and small glands. The mucosa is fibrillar. No sign of a cell-layer.

We must assume that ovulation had taken place at least as early as ten days before examination or even somewhat earlier. The structure of the corpus luteum and the absence of all proliferative changes in the uterine mucosa suggest this conclusion. The new corpora lutea remain small as a result of the insufficient food. The follicles develop only to small-medium size and then undergo atresia. Thus hypotypical ovaries are produced. The uterus is thin, atrophic.

Guinea Pig B.—Examined six days after beginning of feeding experiment; had lost approximately 25 per cent. to 27 per cent. of its weight. This animal was found dead; examination several hours after death.

In the ovaries good-sized follicles with granulosa degeneration; good small and small-medium follicles; various stages of connective tissue atresia. Neither a preserved nor a retrogressing corpus luteum present. The *uterus* is thin with the usual low cylindrical epithelium of surface and small glands. Some epithelial cells at top of papillæ show mucoid transformation; fibrillar mucosa. In this case we have to deal with ovaries which are not yet hypotypical, but which apparently undergo some changes tending towards a hypotypical condition; the larger follicles degenerate and the smaller follicles would in all probability fail to develop to their full size. In this animal ovulation had failed to take place throughout a considerable period of time.

Guinea Pig B_1.—Examined ten days after beginning of feeding experiment; had lost 27 per cent. of its weight. *Ovaries* show

small and small-medium good follicles. In the latter follicles a slow solution of granulosa sets in. We see some follicles of almost medium size without granulosa, but as yet without connective tissue ingrowth. Follicles in various stages of connective tissue atresia, especially numerous follicles in the last stages of connective tissue atresia with relative prominence of the theca interna. A small yellow fibrous body, the remnant of an atretic corpus luteum is present.

Uterus thin with the usual relatively low epithelium and small glands and fibrillar mucosa. In this case after degeneration of the corpus luteum of the former period, a new ovulation failed to take place and the follicles did not grow to normal size. A hypotypical ovary resulted.

Guinea Pig C.—Examined fourteen days after beginning of feeding experiment; had lost 36 per cent. of its weight. *Ovaries* small, living corpora lutea with small center of fibrous tissue. There is perhaps one mitosis in a Lutein cell. In addition there are fibrous, yellow bodies, the remnants of degenerated corpora lutea. Small and small-medium good follicles. Medium follicles show slow granulosa degeneration. The ingrowth of connective tissue into the follicles is probably somewhat delayed; there are a number of follicles with loss of granulosa and as yet without ingrowth of connective tissue. Various stages of connective tissue atresia of follicles are seen. Atretic follicles in the last stage of atresia and with a large mantle of theca interna are frequent. *Uterus* shows the same character as in the other animals; relatively low epithelium, small glands, fibrillar mucosa. Mitoses nowhere visible.

We may assume that in this case the last ovulation took place very soon after the beginning of the feeding experiment. No trace of proliferation was visible in the uterine mucosa. This indicates that at least ten days had elapsed since the last ovulation, a conclusion in accordance with the character of the corpora lutea. The follicles instead of growing to normal size after ovulation, remained small and underwent premature atresia.

Guinea Pig D.—Examined fourteen days after beginning of feeding experiment; had lost 34 per cent. of its weight. *Ovaries* with small and small-medium good follicles. In the latter slow

granulosa degeneration sets in, leading to a complete solution of the granulosa cells with subsequent ingrowth of connective tissue resulting in various stages of connective tissue atresia. Follicles in the last stages of connective tissue atresia form a prominent part of the ovaries. Neither a new corpus luteum nor a remnant of a retrogressing corpus luteum visible. The last ovulation had therefore taken place more than four weeks previous to examination. The *uterus* is thin, with relatively low epithelium and small glands. Much secretion is present in surface epithelium and some gland ducts. The mucosa is thin and fibrillar.

In the four guinea pigs in which ten days or more had elapsed since the beginning of the experiment the ovaries had become hypotypical; while in guinea pig B, which was examined six days after the beginning of the experiment, degenerative changes took place in the large follicles, probably preparatory to the ensuing hypotypical changes. In this animal the relative loss in weight had been almost as marked in six days as in the other guinea pigs in a longer period. It seems therefore that a minimum time has to elapse before the hypotypical condition of the ovaries is established. The latter can be obtained in one of the following two ways: First, after the degeneration of all the good-sized follicles following ovulation, the small follicles fail to reach full size. Before they have grown to medium size, the granulosa cells perish and connective tissue grows in. Second, the large follicles degenerate perhaps at a somewhat accelerated rate without an ovulation having preceded this change, and the small follicles fail to reach medium size. The granulosa of the small-medium follicles does not show the karyorrhexis affecting simultaneously a large number of the granulosa cells and characteristic of the beginning atresia of large follicles; isolated granulosa cells may, however, show karyorrhexis.

In all of the animals with exception of guinea pig *D* ovulation had probably taken place within the last month; and in two animals within the last two weeks preceding the time of examination. We are therefore justified in assuming that at the beginning of the experiment the ovaries had been fully developed in these animals in accordance with their initial weight and age. The lack of a sufficient amount of food however prevented

apparently the corpora lutea from reaching full size. It is furthermore probable that toward the end of the experiment the ingrowth of connective tissue into the cavity of the atretic follicles was somewhat delayed. Still the difference in the behavior of granulosa cells and connective tissue is striking. While the former becomes dissolved as a result of the underfeeding, the latter is still active and organizes the cavity of the follicle. The uterus in all cases was thin, and its mucosa did not show any sign of growth. The ova represent a further element still active under these conditions. In the small-medium follicles in process of atresia mitotic and amitotic divisions of the nuclei take place, as well as a division of the superficial part of the egg into cell-like fragments in which however usually nuclei are not visible.

III. Series. (See Table II.)

Fifteen guinea pigs were used in this series, five, weighing between 534 and 380 grams at the beginning of the experiment, had been bred in Missouri; ten younger ones, varying in weight between 378 and 259 grams, were obtained from Iowa. Three of these animals were well fed during the period of the experiment and served as controls. In addition two other control guinea pigs were examined at the beginning of the experiment. We shall first describe ovaries and uterus of the control animals.

1. *Control Animals.*

Guinea Pig No. 3.—Weight Oct. 18, 474 grams. Nov. 11, twenty-four days after beginning of experiment, weight 613 grams. The animal was found pregnant. The ovaries were normal; they contained good follicles of all sizes, large follicles in granulosa degeneration, good mature follicle as well as mature follicles with ganulosa degeneration. Follicles in various stages of connective tissue atresia. Good corpora lutea as well as degenerated corpora lutea (yellow-fibrous bodies). The *mammary gland* was typically proliferating.

Guinea Pig No. 9.—Weight Oct. 18, 286 grams; weight Nov. 2, 375 grams; examined Nov. 4. *Ovaries:* Good corpora lutea, the cavity of which was filled with connective tissue, retrogressing corpora lutea; good small and medium follicles, various stages

of connective tissue atresia, especially the later stages are well represented. The *uterus* shows a predeciduomatous cell layer with mitoses in the mucosa. The gland fundi with high cylindrical cells. The *mammary gland* consists of acini with relatively high, well-staining epithelium, but without mitoses, and with rather cellular connective tissue between the acini. We have here to deal with normal sexual organs in an animal at a period about five days after ovulation.

Guinea Pig No. 12.—Weight Oct. 18, 263 grams. Weight Nov. 11, 352 grams. In the left horn of the uterus two pregnancies were found. *Ovaries:* Good corpora lutea; their central cavities are filled with connective tissue; good follicles of all sizes. Follicles with granulosa degeneration; good mature follicles; various stages of connective tissue atresia. *Uterus* is that of pregnancy. In addition to these three guinea pigs, two other guinea pigs obtained from Iowa (1*a* and 1*b*) were examined as controls at the beginning of the experiment.

Guinea Pig No. 1a.—Weight Oct. 19, 378 grams. *Ovaries:* Good corpora lutea; the central cavity is almost completely filled by connective tissue, but a small remnant is still left unorganized. Good small and medium and almost large follicles. Some follicles in the last stages of connective tissue atresia. *Uterus* well developed with a predeciduomatous cell layer in mucosa. Fundi of glands with high cylindrical cells. *Mammary gland:* Acini with good cuboidal epithelium and a few mitoses; cellular stroma. We have here to deal with normal sexual organs in an animal about five to six days after ovulation.

Guinea Pig No. 1b.—Weight Oct. 19, 271 grams. *Ovaries:* Good follicles of all sizes, including large and mature follicles. Much hyperemia around the mature follicles, capillaries in theca interna of these follicles much enlarged. Some follicles in various stages of connective tissue atresia, but relatively few follicles in the last stage of connective tissue atresia. No sign of new or degenerating corpora lutea. The *uterus* showed high cylindrical surface epithelium; gland ducts with high epithelium; gland fundi with lower epithelium. The mucosa is hyperemic and succulent and papillomatous. A few mitoses in surface epihelium and at the opening of the glands. *Mammary gland*

very small. A few acini with good cuboidal-cylindrical epithelium with a few mitoses; stroma between acini cellular. The animal was apparently for the first time in her life in the period of heat. We see then that all the control guinea pigs at the beginning and at the end of the experiment were perfectly normal; they were sexually mature and underwent the typical sexual cycle.

2. *Undernourished Animals.*

In order to shorten the description of our findings, we shall from now on designate ovaries in which atresia sets in when the follicles are as yet of small or small to medium size as hypotypical follicles of the first order, and ovaries in which atresia sets in at a slightly more advanced stage of the development of the ovaries, in follicles which have reached or almost reached medium size as hypotypical ovaries of the second order. The uterus is in those cases thin and shows the characteristics described in the fourth series. We shall designate such a uterus as hypotypical.

Guinea Pig No. 1.—Still active at time of examination. Abortion had taken place recently. Ovaries showed corpora lutea with marked vacuolization and thick-walled vessels. The vacuolization affects all parts of the corpus luteum, but especially the periphery. These are the corpora lutea of the preceding pregnancy; following abortion they underwent degenerative changes. Atretic yellow bodies (the remnants of degenerated corpora lutea); good small and medium follicles and only an exceptional good large follicle. The large majority of the large follicles showed granulosa degeneration; follicles in various stages of connective tissue atresia. One almost mature follicle. *Uterus:* Cells of surface epithelium high and large with prominent cytoplasm; glands with rather high epithelium, but with less prominent cytoplasm; many mitoses in surface epithelium; fibrillar mucosa. At one place there are signs that regeneration had taken place and that a decidua had been present. *Mammary Gland:* Large. Acini with good cuboidal epithelium and small lumen. Some colloid in lumen. Some large vacuoles in epithelial cells. No mitoses. Cellular stroma. Some mononu-

clears and polynuclears in stroma. Beginning secretion following abortion. In this case the underfeeding was probably responsible for the abortion. In the controls otherwise held under the same conditions, but given sufficient food, pregnancy proceeded normally. Ovaries, uterus and mammary gland corresponded to the stage of the sexual cycle of this animal.

Guinea Pig No. 2.—Weight Oct. 18, 464 grams; Nov. 18, 302 grams. Following this date it received a full ration of food for a few days and on Nov. 25 it had regained a part of its loss, weighing now 357 grams. Underfeeding was again resumed. On Nov. 30, it weighed 303 grams and it was examined. *Ovaries:* Two young corpora lutea with dilated capillaries. There is in the corpora lutea a central cavity surrounded by a little peripheral connective tissue. Mitoses are found in the capillaries and in the central connective tissue. Atretic yellow bodies with vacuoles and thick vessels are also present. Small and small-medium good follicles with a very thin layer of granulosa which even around the egg is imperfect. Some mitoses in the granulosa cells. In other small-medium follicles granulosa is lost. Follicles in various stages of connective tissue atresia. The *uterus* shows a high cylindrical surface epithelium, glands with cuboidal cells and a very cellular myxoid mucosa. The *mammary gland* consists of good acini without mitoses; it presents an intermediate character. Ovulation had taken place about 2–3½ days previous to the examination. It is probable that following ovulation the ovaries ceased to develop normally and are becoming hypotypical. The solution of granulosa cells in small-medium follicles suggests such an interpretation. We may assume that probably following the transitory gain in weight a few days before examination ovulation occurred. For a considerable period previous to the last ovulation, ovulation had failed to take place, probably in consequence of the underfeeding. We find therefore only remnants of degenerated corpora lutea, representing the corpora lutea of the preceding cycle. The underfeeding did not prevent mitotic division in various structures, nor did it prevent the development of a slightly predeciduomatous condition of the uterine mucosa.

Guinea Pig No. 4.—The animal was in a dying condition,

when examined. Only one *ovary* was examined microscopically. Small and small-medium good follicles; some granulosa degeneration in other small-medium follicles. Various stages of connective tissue atresia; especially many follicles in the last stages of connective tissue atresia, much interstitial gland. No corpus luteum present. *Uterus* showed low cuboidal-cylindrical epithelium of surface and glands without mitoses; fibrillar mucosa. The *mammary gland* consisted of dense fibrous tissue with a few ducts and acini; acini with cuboidal epithelium and without colloid in the lumen. At places the stroma was somewhat more cellular. In this case we can be sure that the ovaries were hypotypical, although only one ovary had been examined. The condition of the uterus makes it certain that a recent ovulation had not taken place. The animal has been markedly affected by the underfeeding, it was in a dying condition at the end of the experiment.

Guinea Pig No. 5.—The animal was found dead. Hypotypical ovaries of the second order. Some medium follicles with granulosa degeneration. Two retrogressing small vacuolar corpora lutea with thick vessels. *Uterus* is hypotypical. In this case ovulation had been delayed.

Guinea Pig No. 6.—Examined in a dying condition. Hypotypical ovaries of the second order. Some almost medium follicles with a thin wall of granulosa. In follicles of almost medium size some granulosa degeneration. No corpus luteum or remnant of a corpus luteum visible. *Uterus* hypotypical.

Guinea Pig No. 7.—Found dead. No noticeable postmortem changes. Hypotypical ovaries of the first order. Small degenerating corpora lutea with vacuolar cells and thick vessels. *Uterus* hypotypical. In this case a new ovulation had not yet taken place, although the corpus luteum of the preceding period was degenerating. The loss of weight in this case had been rapid.

Guinea Pig No. 8.—Found dying. Hypotypical ovaries of the second order. There is present a retrogressing small corpus luteum with vacuolar cells. Uterus hypotypical. Mammary gland shows dense fibrous tissue and small gland ducts without mitoses. Ovulation was delayed.

Guinea Pig No. 10.—Found dead, slight postmortem changes.

TABLE II.

III. SERIES.

Guinea Pig.	Original Weight.	Loss of Weight in Per Cent. of Original Weight.	Period During Which Loss of Weight Occured.	Result.
No. 1..	534 grams	28%	14 days 30% in 24 days	Abortion. Typical cycle, delayed ovulation.
No. 2..	464 grams	23%	33 days 35% in 43 days	Period of refeeding preceded examination. Ovulation with growth in uterine mucosa a few days previous to examination. Ovulation had been suspended for a long period of time. Beginning hypotypical ovaries.
No. 4..	415 grams	31%	14 days	Dying. Hypotypical ovaries of first order.
No. 5..	380 grams	28%	12 days.	Found dead. Hypotypical ovaries of second order. Delayed ovulation.
No. 6..	313 grams	27%	14 days	Dying. Hypotypical ovaries of second order.
No. 7..	302 grams	In 5 days about 9% of the weight lost; had lost some weight in the preceding 10 days	7 days	Hypotypical ovaries of first order. Delayed ovulation.
No. 8..	305 grams	27%	7 days	Dying. Hypotypical ovaries of second order. Delayed ovulation.
No. 10.	292 grams	In 5 days lost 12% of its weight. Died 2 days later	7 days	Found dead. Hypotypical ovaries of first order. Delayed ovulation.
No. 11.	283 grams	26%	7 days	Dying. Hypotypical ovaries of second order. Delayed ovulation.
No. 13.	259 grams	Lost in 5 days 13% of its weight	7 days	Hypotypical ovaries of second order. Delayed ovulation.
		Controls.		
No. 3..	474 grams	29%	24 days	Well-developed pregnancy. Normal ovaries.
No. 9..	286 grams	31%	15 days	5 days after ovulation. Ovulation evidently occurred at normal intervals.
No. 12.	263 grams	34%	24 days	Pregnancy. Normal ovaries.
		Additional Controls; Examined at Beginning of Experiment.		
No. 1a.	370 grams			About 5–6 days after ovulation. Normal ovaries and uterus.
No. 1b.	271 grams			Animal in first heat. Normal sexual organs.

Hypotypical ovaries of the first order. A small vacuolar corpus luteum. Hypotypical uterus. In this case again delayed ovulation.

Guinea Pig No. 11.—Was found dying. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Uterus hypotypical. Delayed ovulation.

Guinea Pig No. 13.—Found dead. Some postmortem changes. Hypotypical ovaries of second order. Small vacuolar retrogressing corpora lutea. Hypotypical uterus.

In this series all the guinea pigs show hypotypical ovaries after a loss of weight of approximately 26–31 per cent. in the course of one to two weeks. In Guinea Pig No. 13 we find in a period of five days a loss of 13 per cent., but in the last two days before death a further considerable loss of weight had in all probability occurred. In 2 cases (Guinea Pigs Nos. 1 and 2) we do not yet find hypotypical ovaries. In the first animal, however, abortion took place toward the end of the experiment. We may attribute the abortion to the lack of a sufficient quantity of food. Abortion is not usual among guinea pigs without a preceding experimental interference. Following the abortion a new ovulation had not yet taken place at the time of examination. In the second animal (Guinea Pig No. 2) ovulation had taken place shortly before the conclusion of the experiment, but there were indications of a beginning hypotypical condition of the ovaries following the ovulation. In these two cases we have in the first place to deal with somewhat heavier animals, and secondly, a slightly smaller relative loss of weight was distributed over a longer period of time. It is probable that the greater initial weight of these guinea pigs caused a greater resistance to the underfeeding; their general condition was less affected by the underfeeding and consequently we find as yet no marked changes in ovaries and uterus. In the five control animals of this series the sexual cycle took its normal course; pregnancy as well as ovulation proceeded normally.

II. Series. (See Table III.)

Five guinea pigs were used in this experiment. Guinea Pig No. 1 served as control, the other four were undernourished and

received only water and a few leaves of grass each day. The experiment began June 30, 1916. For briefness sake, only abstracts of the descriptions can be given.

TABLE III.

II. SERIES.

Guinea Pig.	Original Weight.	Gain or Loss in Weight in Per Cent. of Original Weight.	Duration of Experiment.	Result.
No. I, control.	438 grams	A loss of 15 grams	9 days	At end of pregnancy. Normal ovaries and uterus.
No. II.	462 grams	−29%	9 days	Perhaps first change in direction toward development of hypotypical ovaries. Probably abortion a short time previously. Delayed ovulation.
No. III.	454 grams	−38%	9 days	Not hypotypical ovaries, but beginning change tending in that direction. Delayed ovulation.
No. IV.	428 grams	−35% (in 9 days)	10 days	Not yet hypotypical follicles but probably some retardation in the development of follicles. Delayed ovulation. Animal very weak at end of experiment.
No. V..	439 grams	−34% (in 9 days). Then a gain of 24% of the original weight and of 31% of the lowest weight reached July 9	21 days. (9 days deficient and 11 days full feeding)	No mature follicles and delayed ovulation; ovaries otherwise normal.

Guinea Pig No. 1.—Control. In one horn of uterus one embryo: near end of pregnancy. In this case the condition of *ovaries*, *uterus* and *mammary gland* corresponded to the last stage of pregnancy. In the ovaries large and mature follicles were present. The mammary gland was proliferating.

Guinea Pig No. II.—*Ovaries:* Good small and medium follicles, some larger follicles in granulosa degeneration and perhaps one well preserved large follicle. Most follicles seem to degenerate, when in medium size. Various stages of connective tissue atresia. Large vacuolar corpora lutea with thick vessels. Atretic yellow body (remnant of corpus luteum). *Uterus:* Relatively low cuboidal-cylindrical epithelium of surface and glands. Some

mitoses in surface epithelium, nowhere else; fibrillar mucosa. *Mammary gland:* Large gland, with acini of various sizes, often filled with colloid material. Fibrous or fibrillar stroma, with small nuclei. Some epithelial cells with vacuoles, indicating slight secretory activity. In this case there is perhaps present a beginning of a change in the direction toward the development of hypotypical ovaries; but as yet the ovaries have not reached this condition. The state of the mammary gland and possibly of the ovaries suggests that an abortion has taken place a relatively short time previous to the examination. The large vacuolar corpora lutea with thick vessels, as well as the condition of the mammary gland, which shows signs of secretion, suggest such a conclusion.

Guinea Pig No. III.—Ovaries: Young corpora lutea. Loose central connective tissue fills the cavity. Atretic yellow vacuolar body with thick vessels (remnant of corpus luteum). Good small, medium and almost large follicles. No large follicles, but a few almost large follicles with granulosa degeneration. A number of small-medium or medium follicles lost their granulosa through a gradual process of solution and form cysts in which the egg is lying free. In other degenerating follicles of this kind connective tissue grows into the cavity. Many follicles in last stage of connective tissue atresia. *Mammary gland:* Good sized, acini with cuboidal epithelium, fibrous stroma. No mitoses. The ovaries are not yet hypotypical, but the condition of the smaller follicles indicates a beginning change in this direction.

Guinea Pig No. IV.—Nine days after the beginning of the experiment the animal was offered a larger quantity of food, but refused to eat it. One day later it was found in a very weak condition and it was examined. *Ovaries:* Corpora lutea vacuolar, with thick vessels; they show beginning degeneration, atretic yellow bodies (the remnants of degenerated corpora lutea). Good small and small-medium follicles. Large follicles show granulosa degeneration. Well preserved large follicles are not present. Follicles in various stages of connective tissue atresia. *Uterus:* Relatively low surface epithelium. Similar epithelium in glands. Fibrillar mucosa without mitoses. *Mammary gland:* small gland in a resting condition with marked development of

fibrous tissue. In this case the ovaries are not yet hypotypical, but the lack of good medium and large follicles indicates that some inhibition in the growth of follicles occurred, while the large follicles that had developed previously failed to mature and degenerated.

Guinea Pig No. V.—Weight June 30, 439 grams; July 9, 289 grams. From July 10 on a full ratio of food was given daily and the animal gained in weight. On July 21 it weighed 396 grams; it had not yet regained its original weight. On that date it was examined. *Ovaries:* Contained an atretic yellow body (remnant of a corpus luteum). Good follicles of all sizes, including good large follicles. Large follicles in granulosa degeneration and in various stages of connective tissue atresia. *Mammary gland:* Small intermediate, no mitoses. In this case the ovaries are on the whole normal; but apparently mature follicles did not develop and an ovulation had not taken place for a long period of time previous to the examination, despite the fact that the animal had regained part of its weight.

We find then in this series that hypotypical ovaries did not develop as the result of underfeeding; but in Guinea Pig Nos. II., III., and IV. we find some changes which indicate a tendency toward the development of hypotypical ovaries; either a premature atresia sets in in follicles which are only of medium size, or even smaller, or at least a retardation in the development of large follicles apparently occurred toward the end of the experiment. In all cases mature follicles were absent and ovulation was delayed. In the one animal which received a full ratio of food after a preceding period of underfeeding the follicles likewise failed to mature and ovulation failed to take place during the period in which the animal gained in weight. It is, however, possible that the delay in ovulation was not exclusively due to the undernourishment. In one case abortion had in all probability taken place previous to the examination, probably as the result of the underfeeding. In all these cases the relative and especially the absolute loss of weight had been as great as in the guinea pigs of the III. and IV. series, or it was even greater, and yet hypotypical ovaries had not developed. This result may be due to the greater weight of the animals at the beginning of

the experiment. All the guinea pigs weighed between 400 and 500 grams. These animals were therefore less affected in their general health by the lack of sufficient food than the animals in the other series. Only guinea pig No. IV. showed marked weakness. The duration of the experiment was on the average somewhat shorter than in series III. and IV., and it is possible that this factor also has something to do with the result. But it is probable that the greater weight of the animals in the beginning of the experiment is mainly responsible for the lack of the development of hypotypical ovaries.

First Series of Experiments (See Table IV.).—In this experiment the quantity of food given and changes in weight of the animals had not been determined with the same accuracy as in the later series. While we must consider the possibility of errors in the figures for the weight of the animals it is probable that the error on the whole is not considerable. In most cases the amount of food was increased for several days during the experiment and there was therefore a transitory gain in weight during a certain period. Animals *a*, *b*, *c* and *d* had been thyroidectomized some time previous to the beginning of the experiment; the remaining five animals had been normal.

Guinea Pig No. a.—Ovaries: Atretic yellow bodies (remnants of old corpora lutea). Hypotypical ovaries of second order. Hypotypical uterus. *Mammary gland:* Much gland tissue. Resting, intermediate gland.

Guinea Pig No. b.—March 20, weight 650 grams; April 7, 600 grams; April 30, 280 grams. *Ovaries:* Retrogressing corpora lutea, good follicles of all sizes. Large follicles with granulosa degeneration; follicles in various stages of connective tissue atresia. Not hypotypical ovaries; but no mature follicles developed and ovulation was delayed. *Uterus:* With ordinary surface epithelium and glands. Some vacuoles in surface epithelium. Fibrillar mucosa. Some cystic gland ducts at surface.

Guinea Pig No. c.—March 20, 350 grams; April 7, 275 grams; April 11, 175 grams. Found dead. *Ovaries:* With atretic yellow bodies. Hypotypical ovaries of first order. Hypotypical uterus.

Guinea Pig No. d.—Weight March 20, 500 grams. Receives

full ratio of food; but loses some weight. April 7, 475 grams; April 10, 400 grams, found dead. Had probably been sick. *Ovaries:* Vacuolar corpus luteum with thick vessels. Small medium and almost large food follicles; some small to medium follicles apparently begin to show connective tissue atresia. *Uterus:* Quiescent with follicular mucosa. *Mammary gland:* Large, resting-intermediate. In this case the loss in weight in the animal caused a condition in the ovaries which is approaching the hypotypical state; there are, however, as yet present some slmost large follicles.

TABLE IV.

I. SERIES.

Guinea Pig.	Original Weight.	Change in Weight in Per Cent. of Original Weight.	Duration of Experiment.	Result.
No. *a*..	700 grams	−46%	24 days	Found dead. Hypotypical ovaries of second order. No maturation of follicles. No ovulation.
No. *b*..	650 grams	−57% (In 23 days −53%)	41 days	Killed. Not hypotypical ovaries. No mature follicles; retrogressing corpora lutea. Delayed ovulation.
No. *c*..	350 grams	−50% (In 4 days 21% loss of weight)	22 days	Found dead. Hypotypical ovaries of first order. No new ovulation.
No. *d*..	500 grams	−20% (In 3 days 15% loss of weight)	16 days	Imperfect control. Found dead. Receives a larger quantity of food; almost large follicles in ovaries but some small-medium follicles undergo connective tissue atresia.
No. *e*..	367 grams	Loses 100 grams (27%) in 1st two weeks, then regains weight	38 days	Control; full ratio of food. About 4½ days after ovulation. Normal cycle took place.
No. *f*..	330 grams	−45%	14 days	Died. Hypotypical ovaries of first order. No new ovulation.
No. *g*..	About 420 grams	−58%	17 days	Died. Hypotypical ovaries of first order. No new ovulation.
No. *h*..	About 420 grams	−58%	21 days	Hypotypical ovaries of second order. No new ovulation.
No. *i*..	About 360 grams	−58%	37 days	Hypotypical ovaries of second order. No new ovulation.

Guinea Pig No. e.—Control. Weight March 24, about 365 grams. April 7, 260 grams; received full ratio. May 1, 359

grams; examined. *Ovaries:* Two young corpora lutea with capillaries, with mitoses in lutein and endothelial cells. Atretic yellow bodies. Good small and small-medium follicles; follicles in medium and late connective tissue atresia. *Uterus:* Epithelium in glands high; frequent mitoses in gland fundi. In mucosa predecidual cell layer with frequent mitoses. *Mammary gland:* Large ducts, acini with rather high cuboidal epithelium, very cellular, not fibrous stroma; probably mitoses in acini. This is an animal at a period about 4½ days after ovulation. Mature follicles developed and ovulation took place.

Guinea Pig No. f.—Hypotypical ovaries of first order with small, vacuolar, retrogressing corpus luteum with thick vessels. Hypotypical uterus with a few mitoses in gland ducts.

Guinea Pig No. g.—Hypotypical ovaries of first order; corpus luteum with vacuolar cells and dense fibrous nucleus; probably 15–20 days old. Atretic yellow bodies. Hypotypical uterus. Ovulation had taken place approximately at the time of the beginning of the experiment. The atretic yellow bodies indicate that previous to the experiment the sexual cycle had taken its normal course.

Guinea Pig No. h.—Weight March 24, about 420 grams. April 7, weight 220 grams. April 13, 175 grams. Killed April 14. *Ovaries:* Atretic yellow bodies (remnants of corpora lutea); small and small-medium good follicles. In the small-medium follicles atresia sets in and connective tissue grows into the cavity. A few follicles reach almost medium size and then disintegration of the granulosa and connective tissue atresia set in. Only exceptionally a good medium sized follicle present. Hypotypical uterus; some mitoses in surface epithelium. *Mammary gland:* large. Small acini with or without colloid, with low or medium-sized epithelium and partly vacuolar cells. Stroma fibrillar or fibrous. There may have been secretion in this gland previous to the beginning of the experiment.

Guinea Pig No. i.—Hypotypical ovaries of second order with atretic yellow bodies. Hypotypical uterus; some mitoses in surface epithelium. Guinea pigs *h* and *i* were evidently not so strongly affected by lack of food. They did not die spontaneously at the end of the experiment.

From the results of this series, we may draw the following conclusions: Of the seven animals which were subjected to the low diet, 3 showed hypotypical ovaries of the first order; 3 showed hypotypical ovaries of the second order. In all of these cases ovulation had been prevented. In one case, the ovaries were not hypotypical, although the loss in weight had been just as considerable as in other cases. In this case, however, maturation of follicles and ovulation failed to take place. If we inquire into the condition responsible for these differences in the behavior of different animals, we may suggest that it depends in all probability on differences in the general effect of underfeeding on the animals. Those animals that were alive at the end of the experiment showed a less far-going hypotypical condition than those that had just died at the time of examination. This series shows furthermore that a hypotypical condition may be produced even in a relatively old guinea pig with a considerable initial weight, provided there is a considerable loss of weight extending over a relatively long period of time and a marked influence in the general condition of the animal. If, on the other hand, an animal with a considerable initial weight is less affected generally and its strength is better preserved, throughout the experiment, merely maturation of the follicles and ovulation are prevented from taking place, but a hypotypical condition is not produced. In all those cases in which the ovaries are hypotypical the uterus is thin and shows the resting condition found also in the other series. In this series the loss of weight of the underfed guinea pigs was more considerable than in the other series and the experiment extended over a longer period of time. In the two control animals a hypotypical condition of the ovaries was not found. In one of these the cycle in ovaries and uterus took its normal course; in the other, a loss of weight had occurred, the cause of which remained obscure. While in this case the ovaries do not show a hypotypical condition, some retardation in the development of the follicles had occurred.

Conclusions.

We used in our experiments altogether thirty-four guinea pigs, eight of which served as controls, while twenty-six were sub-

jected to insufficient feeding extending over periods varying between 33 and 6 days. In the majority of experiments the loss in weight varied between 25 per cent. and 35 per cent. of the initial weight. In some cases the loss was greater, in others less. In the controls there was usually a gain in weight during the experiment, but in two cases there was some loss and in one the loss was as much as 20 per cent. of the initial weight. This animal cannot be considered as a perfect control. In the controls the sexual cycle took its normal course and ovaries and uterus were normal in seven animals. In three of these pregnancy was present which proceeded normally. In several others ovulation occurred during the time of the experiment and the follicles developed in a healthy manner. In the one case, in which the animal lost 20 per cent. of its weight, the ovaries were not yet hypotypical, but some change was noticeable tending in that direction. Of the animals subjected to lack of a sufficient quantity of food the ovaries were hypotypical in eighteen cases. There were differences in the degree in which this condition had developed; while the ovaries of some showed a hypotypical condition of the first order, in others hypotypical ovaries of the second order were present. In the former the development of the follicles proceeded only to that stage in which the follicles were of small-medium size. At this stage atresia set in, while in the second kind a few follicles developed to medium or almost medium size; while others became already atretic at an earlier stage. In no case were large follicles produced in any of these ovaries. In three other cases changes were noticeable in the ovaries which suggested a beginning in the direction toward the development of hypotypical ovaries, while in five animals hypotypical changes were not yet noticeable, but an inhibition of ovulation had occurred owing to the fact that the large follicles failed to mature. We see then that in all the animals a failure of the follicles to mature occurred and in 69 per cent. of the animals the ovaries were hypotypical. In addition pregnancy was found in none of the animals. In one of them abortion had occurred, with certainty and in a second one with great probability, in both cases probably as a result of the underfeeding.

If we now inquire into the causes of the variability in the

effects of the underfeeding on the ovaries, our experiments suggest very strongly that in addition to the relative loss in weight two other factors may be of significance, viz., first the length of time during which the loss of weight occurs. It seems that more than six days must elapse, before hypotypical ovaries are found. And secondly, the weight of the animal at the beginning of the experiment is of importance. The more the animal approaches full size, the greater is the difficulty with which the changes in the ovaries set in. They are more readily produced in animals weighing between 300 and 400 grams than in animals weighing over 400 grams. But they can be produced in the latter. The effects on the ovaries are therefore to some extent parallel to the effects of undernourishment on the general condition of the animals. Heavier guinea pigs who suffer the same percentage in loss of weight as smaller animals are apparently very much less affected by it as far as their general health is concerned. It is probable that the greater the portion of the food intake is which the animals have to set apart for growth, the more the portion to be used for maintenance is diminished and it seems furthermore that the effect on the general health and on the condition of the ovaries depends upon the portion available for maintenance. The state of the ovaries determines the condition of the uterus, which is always in a resting, almost atrophic condition in cases in which the ovaries are hypotypical. In a similar manner the condition of the mammary gland depends upon the state of the ovaries. We see then that as a result of underfeeding there takes place an increased destruction of granulosa cells which affects even follicles of small or small-medium size. This degeneration of the granulosa cells at such an early stage of the development of the follicles does not occur in the form of a massive destruction, but here and there cells in the rows of the granulosa adjoining the cavity of the follicles are dissolved. While we may notice cells in which the nuclei undergo karyorrhexis, it is probable that other cells perish without a distinct karyorrhexis taking place. If, on the other hand, a degeneration sets in in the larger follicles in normal ovaries, a large number of granulosa cells are destroyed simultaneously and the nuclei of all these cells show karyorrhexis.

We must therefore assume that if the amount of available food

stuffs is diminished below a certain quantity, those cells perish first which are farthest removed from the source of nourishment which is furnished by the capillaries of the theca interna. But the destruction is not limited to the cells adjoining the cavity, but affects in the end the whole granulosa. We see then that under the condition of lack of proper nourishment, a condition which otherwise would occur much later, is observed at an early period of the development of the follicles, and the process of destruction in this case takes place more slowly than in the case of atresia of the large follicles. It is in the small follicles a more chronic, in the large follicles a sudden, acute process. Those granulosa cells in the small and small-medium follicles which survive for a certain time show approximately the same proliferative energy as the granulosa cells of similar follicles in normal ovaries as the investigations of L. S. N. Walsh have shown.[1] We must therefore assume that the remaining cells are fairly healthy and respond to the stimulus to multiply in a way similar to the granulosa cells in normal follicles, while those cells that are markedly affected by the lack of nourishment become dissolved. We have here to deal with a phenomenon similar to the one which we observed in the case of stationary or retrogressing tumors. In our experimental analysis of tumor growth we noticed that in tumors that had ceased to grow mitoses could still be found rather frequently during a certain period following cessation of growth. This observation led us to the conclusion that the stationary or retrogressing condition was brought about not so much through a complete cessation of growth, as through an increased destruction or solution of cells.[2] We see that in the hypotypical follicles the smallest follicles resist, while in the follicles in which a cavity begins to form processes of degeneration set in. We observe the same phenomenon in the destruction of follicles which takes place at the time of ovulation. Here also the largest follicles perish first and the smallest follicles are most resistant. We must assume either that with the growth of the follicles the granulosa cells undergo changes in their constitution, which makes them more sensitive to injurious

[1] Walsh, L. S. N., *Journal Exp. Medicine*, 1917.

[2] Loeb, Leo, *Virchow's Archiv*, 1902, CLXVII., p. 175; 1903, CLXXII., 345; also E. P. Carson White and Leo Loeb, *Centralbl. f. Bact.*, 1910, LVI., 488.

influences, or that with the growth of the follicles the nourishment becomes more difficult, and that this unfavorable state of the cells makes them more vulnerable, if injurious conditions arise. In case the second interpretation should be correct we would have to assume that the process of degeneration leads auto-katalytically to further destruction of the follicles of the remaining granulosa cells.

These observations lead furthermore to the conclusion that the formative stimuli, which call forth cell proliferation, are not identical with the food stuffs on which ultimately the life of the cells depends and which are therefore necessary for cell multiplication, a conclusion in full accord with other facts which may be discussed in another connection. Our previous observations and especially the additional observations of Walsh enable us to state the character of the formative stimulus which acts on the granulosa cells. As far as we can determine this stimulus emanates from the egg, and affects principally those granulosa cells nearest the egg, but to a less extent also the more distant cells. It is probable that this stimulus increases simultaneously with the growth of the follicles from small to medium size. In the medium-sized follicles it reaches a maximum. From now on certain unfavorable factors begin to make themselves felt with increasing severity and they lead ultimately either to a degeneration of the granulosa or to that increased differentiation which is characteristic of maturation of the follicles. Perhaps it is the difficulty in the nourishment of the granulosa—a difficulty which increases with the further enlargement of the medium follicles—which is the factor which counteracts the cumulative action of the ovum and prevents a steady increase in proliferative power of the granulosa cells, and which ultimately leads to their death. Thus may be explained the typical growth curve consisting of an ascending and a descending branch as has been described by Walsh. As we stated above, the ova in the follicles which in the hypotypical ovary undergo a premature atresia, show progressive changes. Thus the same factor that causes destruction of the granulosa cells acts on the eggs as a stimulus. In a similar manner connective tissue cells show activity, migrate into the follicular cavity and thus contribute to the atresia of follicles under conditions in which the granulosa cells perish in

consequence of the lack of proper nourishment. We may then conclude that the connective tissue is more resistant to withdrawal of food than the granulosa cells; and this is a conclusion which is borne out by the behavior of the connective tissue under other conditions. This relatively great resistance to lack of food is one of the most important characteristics of the connective tissue which leads to interesting consequences under normal and pathological conditions. While on the whole the connective tissue cells are more resistant than the granulosa cells yet we noticed that under the conditions of ill nourishment prevailing under our experimental conditions, the ingrowth of connective tissue into the follicular cavities was not rarely somewhat retarded. In such cases we see the follicle forming a cyst lined by theca interna in which the ovum lies free or almost free and in which connective tissue has not yet grown.

The atresia of follicles is comparable to a condition which in other organs is designated as cirrhosis. In the case of the atresia of follicles this cirrhotic process is a substitutive one; the stimulus which induces the connective tissue to become active consists in the loss of the granulosa cells which formerly covered the theca interna. As we pointed out on former occasions, we have reason to assume that there exist in addition stimuli of a different character which call forth cirrhotic processes.[1]

From our investigations it follows that in the guinea pig underfeeding prevents maturation of the follicles and thus causes sterility which lasts as long as the effect of the underfeeding is present in the ovary. This lack of maturation we found in all our cases. In addition we found in the large majority of those animals which had become more markedly affected by the lack of sufficient food, especially in those which had not yet reached their full size, a much farther-going deficiency in the development of the follicles, a condition of the ovaries to which we applied the name "hypotypical." The latter state is altogether incompatible with fertility. The problem that is as yet unsolved concerns the length of time during which this condition is present, and whether the ovaries resume their normal life very soon after the quantity of food has again been increased, or whether the hypotypical condition and lack of maturation

[1] Loeb, Leo, *Journ. Amer. Med. Ass'n*, 1915, LXIV., 726.

persists at least for some time after the resumption of feeding. Further investigations will have to solve this problem. Our results may perhaps also have some bearing on the condition of the ovaries during pregnancy. We have shown that in guinea pigs ovulation does not occur during pregnancy unless the corpora lutea have been extirpated. In other species however, and especially in man it seems that during pregnancy a maturation of the follicles is lacking. Our experiments suggest that this may depend upon a relative insufficiency of food available for the ovaries during pregnancy; thus an approach to a hypotypical condition of the ovaries would be produced.

Summary.

Underfeeding, if very pronounced, prevents maturation of the follicles in the ovaries of the guinea pigs in all cases and in the large majority of cases leads to the production of hypotypical ovaries in which atresia of follicles sets in before the follicles have reached medium size. Underfeeding leads to a premature solution of granulosa cells. Connective tissue is more resistant to lack of food than the granulosa. The *uterus* in cases of underfeeding is in a resting or atrophic condition. Thus underfeeding produces at least temporary sterility. Those cells farthest removed from the blood vessels suffer first as the result of underfeeding, die and become dissolved, while in those granulosa cells which remain alive the growth stimulus which in part at least emanates from the ovum causes for a short time a normal cell proliferation. Just as the underfeeding produces more pronounced general effects in younger animals, the effect on the ovaries is likewise more marked in younger animals. Through underfeeding it is, however, possible to produce a hypotypical condition even in old guinea pigs. In those animals which had been pregnant at the beginning of the experiment underfeeding led to abortion.

There exists a noteworthy analogy in the relation of cell proliferation to cell destruction in the hypotypical ovaries and in stationary or retrogressing tumors.

Our results emphasize the distinction between food stuffs and proliferative cell stimuli and they may in addition throw light on the condition of the ovaries during pregnancy.